TITLE: GENETIC MANIPULATION OF SOME AROMATIC AND FLAVOR CONSTITUENTS OF FLUE-CURED TOBACCO

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ABSTRACT: Leaf carotenoids are important precursors of aromaticity and flavor in tobacco. The feasibility of using total carotenoid concentration in cured leaf as an indirect selection criterion for genetically altering aroma and flavor constituents was examined. Five cycles of mass selection for high and low carotenoid concentrations in cured leaf were completed using an $F_2$ population of flue-cured cultivars 'Hicks' x 'Coker 139' as the initial selection base. Evaluation of the 5 mass selection cycles showed that the high and low populations had diverged 41% for total carotenoids, based on a linear regression model. The economically important characters total alkaloids and total nitrogen were positively correlated with carotenoid changes and diverged 46% and 11%, respectively; reducing sugars were negatively correlated with carotenoid changes with a divergence between high and low populations of 15%. Leaf yields and grade index were negatively correlated with carotenoid changes. The influence of carotenoid and other chemical constituent changes on the aroma and flavor of flue-cured tobacco was confirmed in a gas-liquid-chromatography study of essential oils. The high population had 35% more flavor and aroma related constituents than did the low population. Cured leaf from the fourth cycle high carotenoid population provided acceptable smoke flavor while the low population was unacceptable.

REVIEW: Traditional tobacco breeding experiments have been directed at the following criteria (in order of importance): 1) disease resistance; 2) agronomic performance; 3) leaf chemistry; 4) physical appearance of cured leaf; and 5) smoke flavor. From results of breeding programs, the hypothesis was advanced that leaf carotenoids, important contributors to flavor, are under genetic control and that carotenoid degradation is non-enzymatic. This paper, based in part on work reported in a thesis by R. A. Beatson, described the results of a study of genetic manipulation to produce an acceptable leaf enriched in carotenoid degradation products.

Hicks broad leaf (high in carotenoids) was crossed with Coker 139 (low in carotenoids with high yield of leaf) to give the $F_1$ generation. Reciprocal crosses of this generation gave $F_2$. The $F_2$ plants were topped but not suckered. Leaf was collected from the bottom, middle and top of the plant. A portion was freeze-dried, ground and analyzed while the remainder was flue-cured before analysis. There was no significant correlation of genotype with stalk position of the leaf but there was a significant genetic variability among plants of this generation. The carotenoid levels were highly correlated between green and cured leaf, and the levels decreased with ascending stalk position. From carotenoid analyses of leaf from each plant of the $F_2$ generation, 10 plants with the highest level were crossed with 10 plants with the lowest concentrations. This procedure was carried through 5 cycles and seed from each cycle planted. Leaf from mature plants of each cycle were then analyzed. Carotenoids in the last cycle had reached 24 mg/100g at the high concentration and 17.8 mg/100g at the low concentration. Alkaloids and total nitrogen showed similar trends. However, there was a reversal in reducing sugars, grade index and leaf yield. These three parameters were higher in the leaf with low carotenoid levels.

Since flue-cured leaf is marketed on the basis of appearance, the leaf that is high in carotenoids (i.e., most flavorful) would bring a lower price in the market because it would be judged of low quality based on its appearance. From this point of view, the genetic manipulation was a failure. However, from a tobacco buyer's point of view, the opportunity to obtain an enriched flavor leaf at a low price certainly is attractive. The high carotenoid genotype developed in this study might be grown outside the allotment program.

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